# United States Department of Agriculture
# Center for Veterinary Biologics
# Testing Protocol

## SAM 408

## Supplemental Assay Method for Titrating Tissue Culture Adapted Vaccine Strains of Infectious Bursal Disease Virus

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**Supplemental Assay Method for Titrating Tissue Culture Adapted Vaccine Strains of Infectious Bursal Disease Virus**

## 1. Introduction

This Supplemental Assay Method (SAM) describes the titration of vaccine strains of tissue culture adapted infectious bursal disease virus (IBDV) in primary chick embryo fibroblast cell cultures (1°CEF).

## 2. Materials

### 2.1 Equipment/instrumentation

Equivalent equipment or instrumentation may be substituted for any brand name listed below.

**2.1.1** Water-jacketed incubator with a humidified 5 ± 1% $CO_2$ atmosphere and temperature set at 37º± 1°C (Forma **Scientific**, Model No. 3158)

**2.1.2** Vortex mixer (Thermolyne Maxi Mix II, Model No. M37615)

**2.1.3** Microliter pipette (Rainin Pipetman, P1000)

**2.1.4** Laminar Flow Biological Safety Cabinet (NuAire Inc., Labgard)

### 2.2 Reagents/supplies

Equivalent reagents or supplies may be substituted for any brand name listed below. All reagents and supplies must be sterile.

**2.2.1** 24-well tissue culture treated plates, planted with 1 ml of 1°CEFs suspension per well at a concentration of approximately 400,000 cells/ml. 1°CEFs are from susceptible specific pathogen free (SPF) embryonating chicken eggs.

**2.2.2** M-199/F-10 maintenance media

**2.2.3** Sterile distilled or deionized water, 100-ml aliquots in 100-ml serum vials with rubber stoppers and aluminum caps

**2.2.4** Needle, 1 1/2-inch x 18-gauge

**2.2.5** LuerLok disposable syringe, 5- or 10-cc

**2.2.6** Sterile pipette tips, Rainin 100-1000

**2.2.7** Sterile glass test tubes, 16 x 125-mm

**2.2.8** 24-well tissue culture treated plate

**2.2.9** Fetal Bovine Serum (FBS)

**2.2.10** L-Glutamine

**2.2.11** Pipette tips

**2.2.12** Solution

Solution is filter sterilized.

**1.** Maintenance Medium

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|---|---|
| Medium 199 (with Earles salts) (powdered) | 9.9 g |
| Nutrient Mixture F1O (powdered) | 9.8 g |
| Bacto Tryptose Phosphate Broth (powder) | 2.95 g |
| $NaHCO_3$ | 2.5 g |
| Penicillin (potassium G) | 100,000 units |
| Streptomycin | 200 mg |
| HEPES | 5.98 g |
| Fetal Calf Serum (gamma-irradiated) | 1-3% |
| q.s. with distilled or deionized water | 1.0 L |

Adjust pH to 7.35 to 7.4 by adding $NaHCO_3$ solution.

Before use, add 1.0 ml of a 200-mM concentration of L-glutamine per 100 ml medium.

## 3. Preparation for the test

### 3.1 Personnel qualifications/training

Personnel must have experience or training in this protocol. This includes knowledge of aseptic biological laboratory techniques and preparation, proper handling and disposal of biological agents, reagents, tissue culture samples, and

chemicals. Personnel must also have knowledge of safe operating procedures and policies and Quality Assurance (QA) guidelines of the Center for Veterinary Biologics (CVB) or equivalent; and training in the operation of the necessary laboratory equipment listed in **part 2.1**.

### 3.2 Preparation of equipment/instrumentation

Operate all equipment/instrumentation according to manufacturers' instructions and monitor in compliance with current corresponding CVB/National Veterinary Services Laboratories (NVSL) Standard Operating Procedures (SOPs) or equivalent.

### 3.3 Preparation of reagents/control procedures

Prepare reference viruses in the same manner as sample preparation.

### 3.4 Preparation of the sample

#### 3.4.1 Preparation of vaccine for titration

Rehydrate vaccine in 100 ml of sterile purified water. Mix thoroughly. If necessary, further dilute the vaccine so that 1 dose is contained in a volume of 0.1 ml.

## 4. Performance of the test

### 4.1 Preparing dilutions and inoculating plates

Prepare dilution blanks of maintenance medium for the vaccine and positive reference virus. Make tenfold serial dilutions of the vaccine and the positive reference virus encompassing the range of the expected titer. Use at least 4 dilutions for a titration. Inoculate 5 wells with 0.1 ml for each dilution. The sixth well of each row remains uninoculated and serves as a cell control. Incubate in a humidified atmosphere of approximately 5 ± 1% $CO_2$ at 37°± 1°C.

## 5. Interpretation of the test results

### 5.1 Controls

The titer of the positive reference must be within the established range for the test results to be valid. Uninoculated negative control cells are maintained to monitor the integrity of the cell culture system.

### 5.2 Calculating the titer

Microscopically examine the plates daily and track the development of typical IBDV cytopathology including refractile cells. The control wells must remain normal throughout the test. On day 7 postinoculation, calculate the 50% endpoint of infectivity using the Reed-Muench method. This value will represent the titer per dose.

### 5.3 Retests

Conduct retests as required by the Code of Federal Regulations, Title 9 (9 CFR), part 113.8(b) and requirements of minimum release in firm's current Outline of Production, Part V.

### 5.4 Evaluation of test results

**5.4.1** The 9 CFR 113.8(b) defines the criteria for a satisfactory/unsatisfactory serial.

**5.4.2** The firm's requirements of minimum release/stability titers for each IBD vaccine are listed in the current Outline of Production, Part V, for the specific product code.

## 6. Report of test results

Titers are reported out as Tissue Culture Infective Dose 50% Endpoint ($TCID_{50}$) per bird dose. Report the results as satisfactory or unsatisfactory.

## 7. Summary of revisions

This document was rewritten to clarify practices currently in use an the Center for Veterinary Biologics and to provide additional detail. While no significant changes were made that impact the outcome of the test, the following changes were made to the document:

- The introduction has been revised for clarity.
- **2.1** Many items listed were unnecessary to list and have been removed from the document for clarity.
- **2.2** Changes in the reagents/supplies used have been made to the items previously listed for clarity.
- **2.2** The Dulbecco's PBS and trypsin solution (0.25%) have been removed. The growth medium has been removed and replaced with a maintenance medium.
- **2.2.16** The section on cell cultures has been deleted from the document.
- **4.1** The use of the maintenance medium for the vaccine and positive reference virus has been added for clarity.
- **5.2** This section has been revised for clarity.
- **6.** This section has been revised to provide clarification of reporting test results.